Sorting by pools

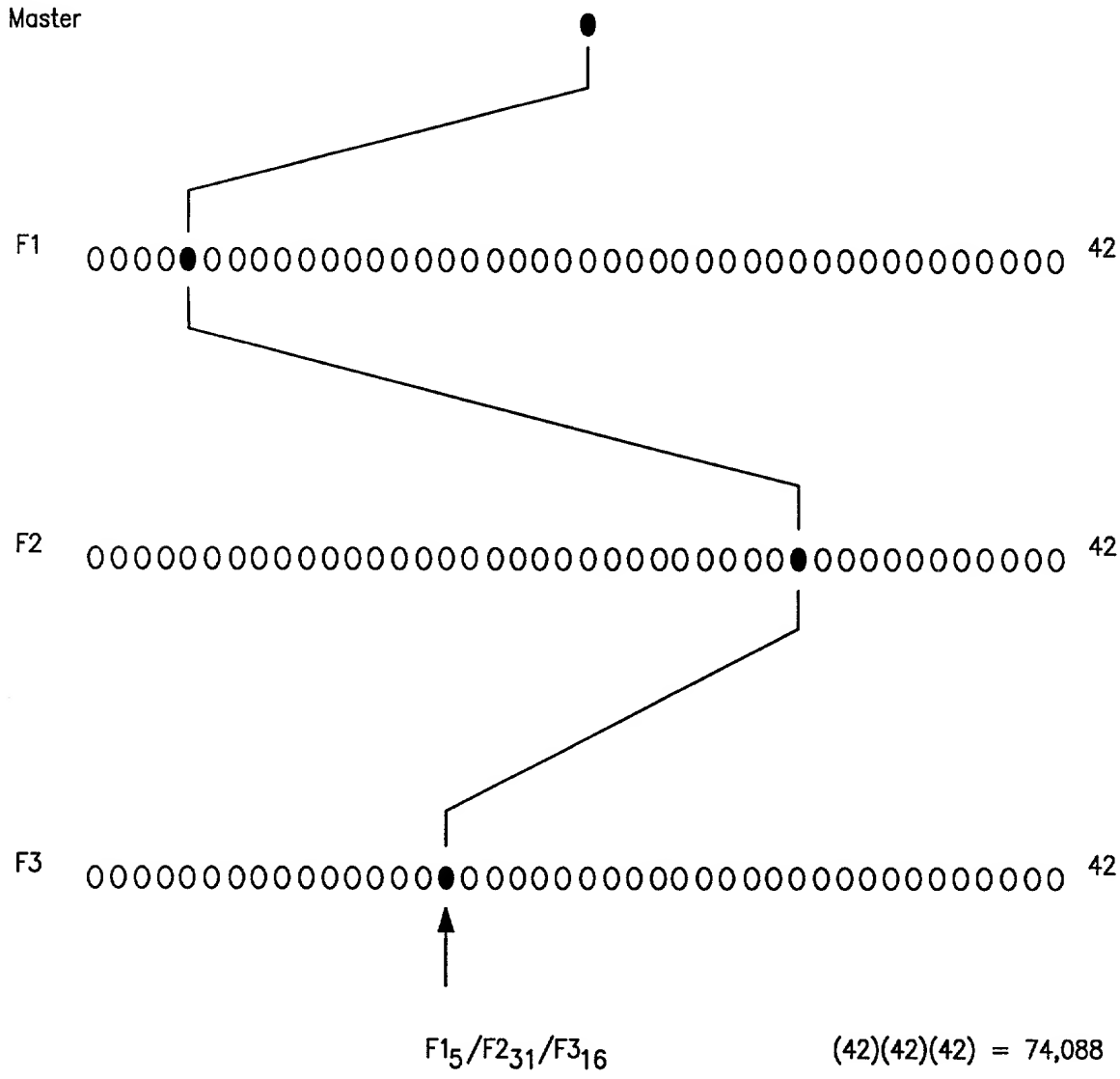


FIG. 1

Sorting by pools: Decreasing pool diversities

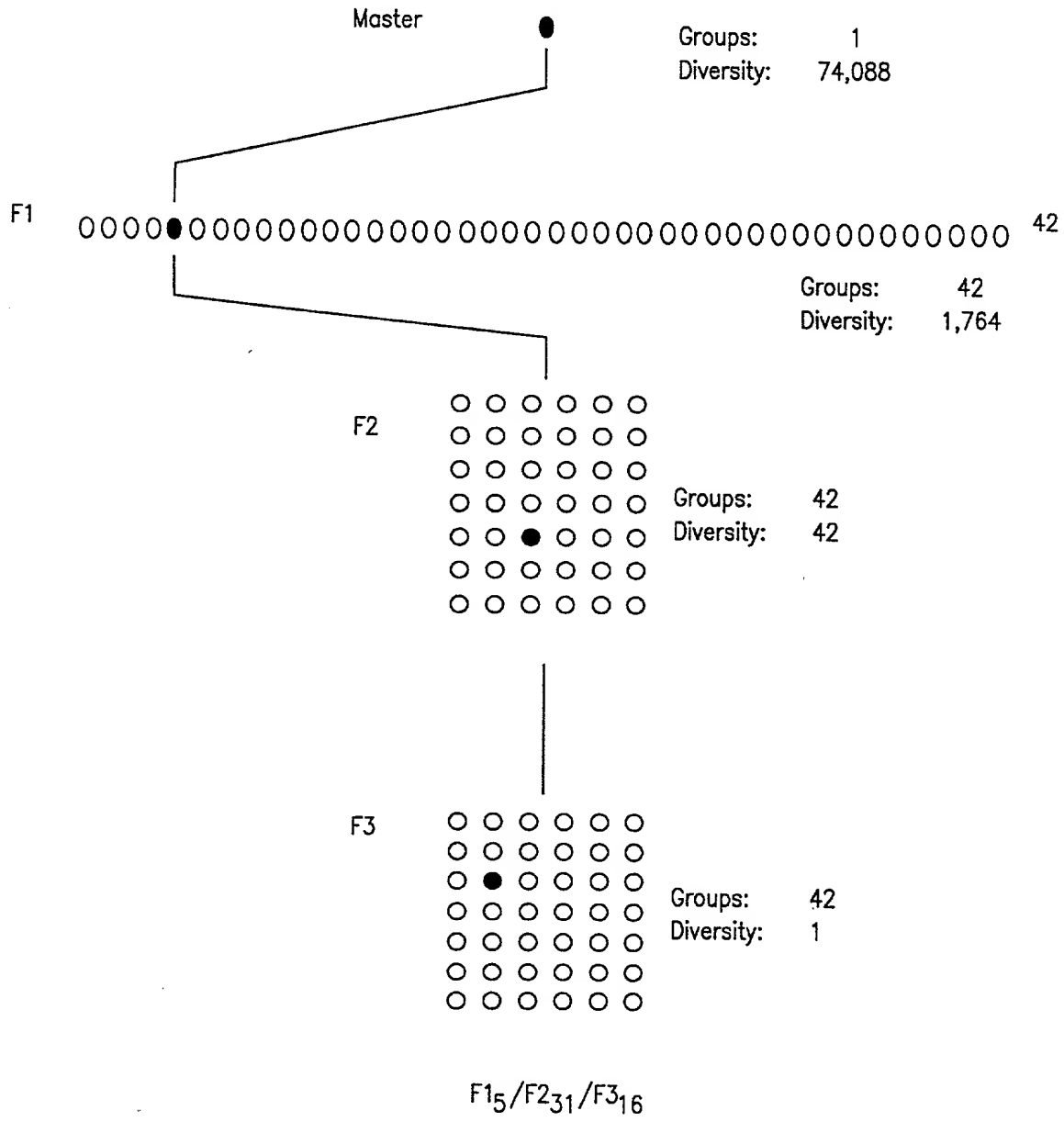
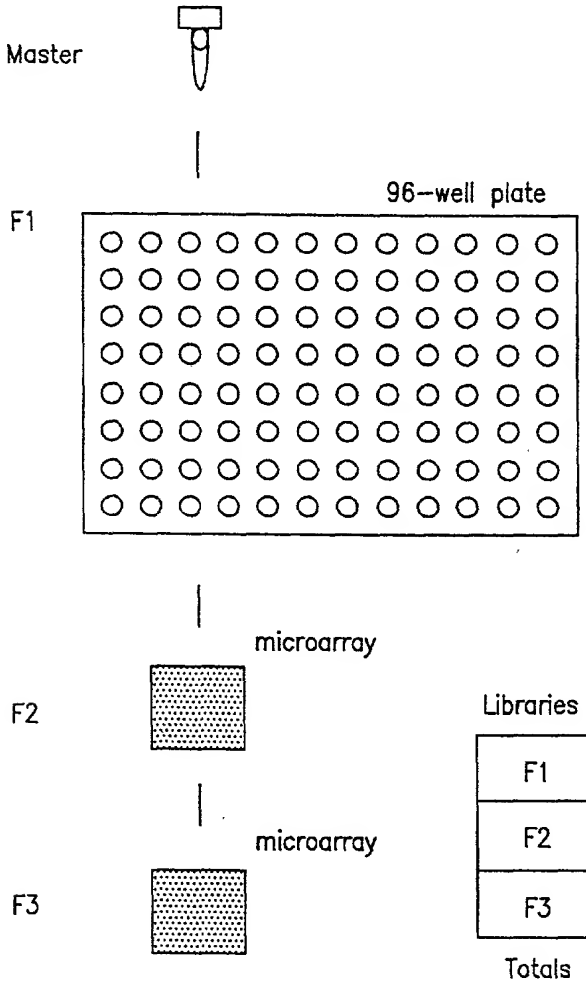


FIG. 2

Sorting by pools: Screening large diversity libraries



Libraries	Pools			
F1	100	1,000	100	1,000
F2	100	100	1,000	1,000
F3	100	100	1,000	1,000
Totals	10^6	10^7	10^8	10^9

FIG. 3

Searching a mutation library

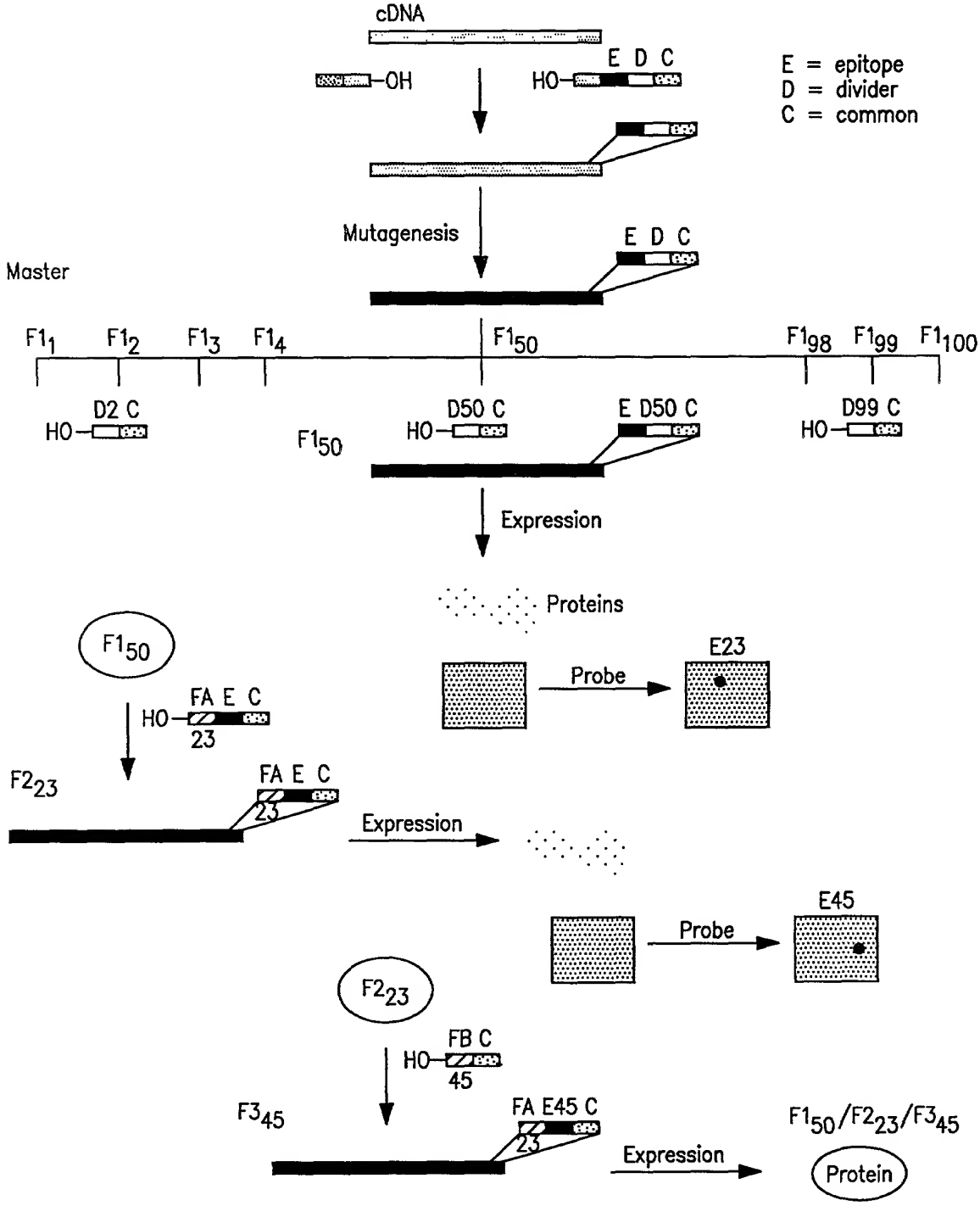


FIG. 4

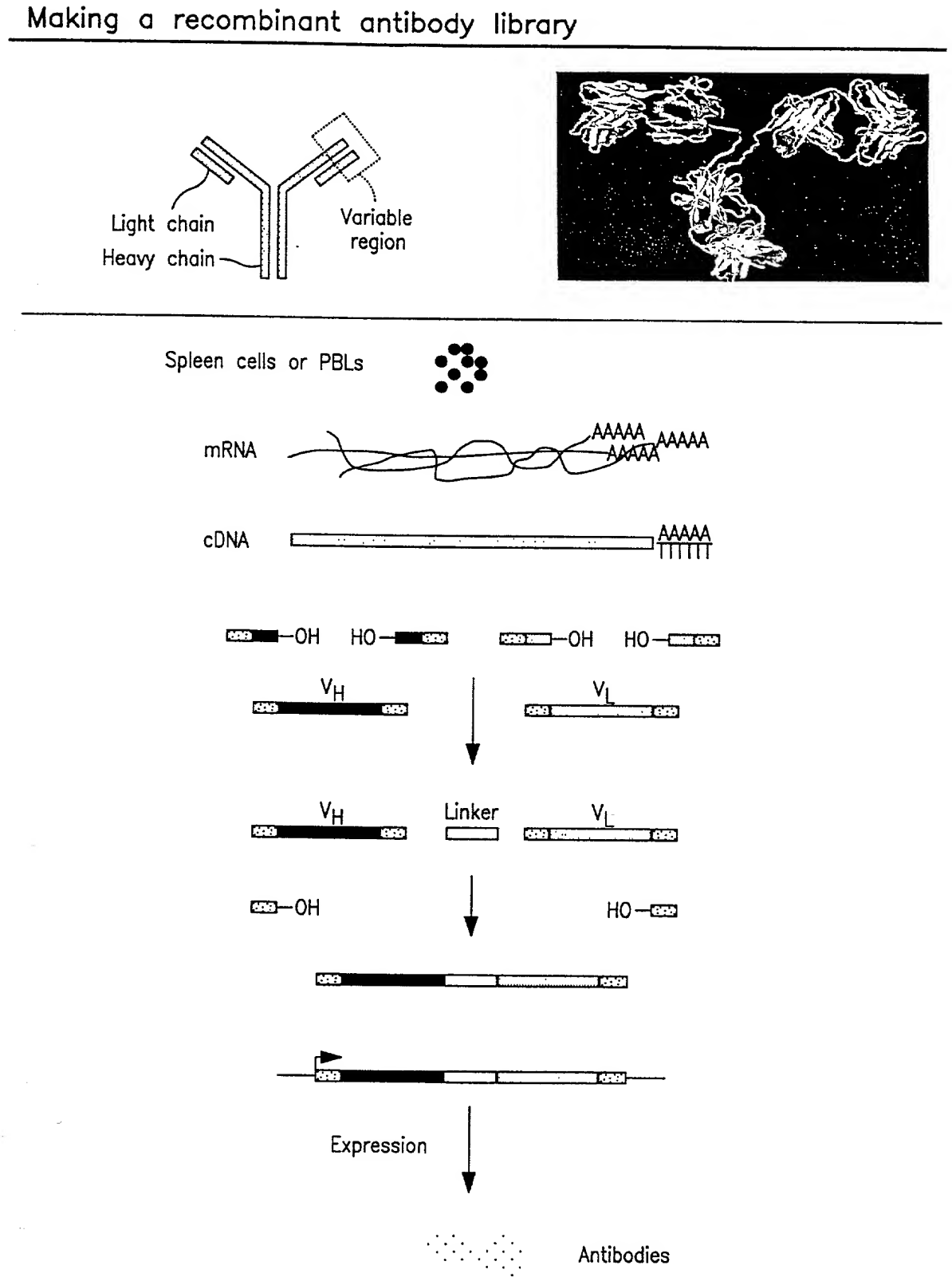
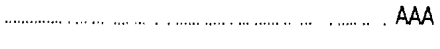


FIG. 5

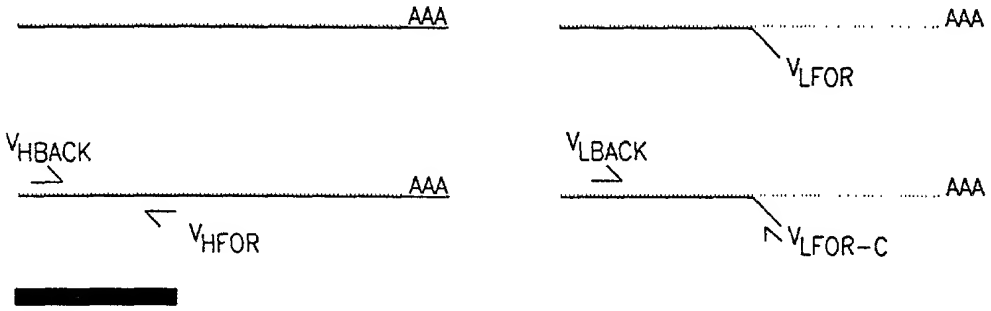
Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs

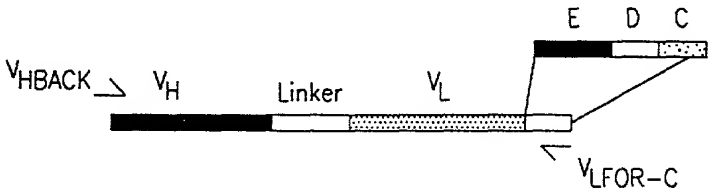


2. cDNA synthesis

3. amplification



4. assembly



V_H Primers		V_L Primers	
Oligo dT	HO–TTTTTTTT(T)$_n$ 3' 5'	V_{LFOR}	J_{kappa} for E D C HO– 3' 5'
V_{HBACK}	V_H back –OH 5' 3'	V_{LBACK}	V_{kappa} back –OH 5' 3'
V_{HFOR}	J_H for OH– 3' 5'	V_{LFOR-C}	C HO– 3' 5'

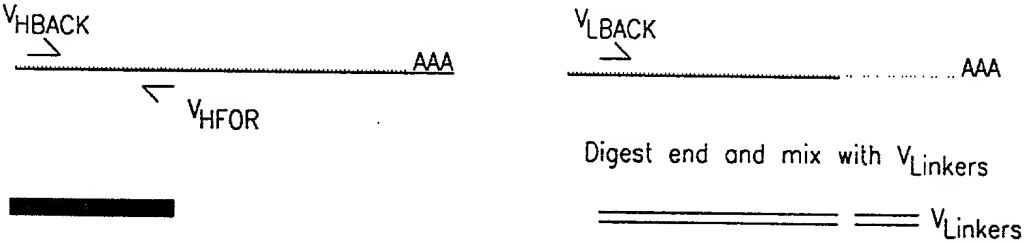
FIG. 6

Creating the master antibody library: Linker addition

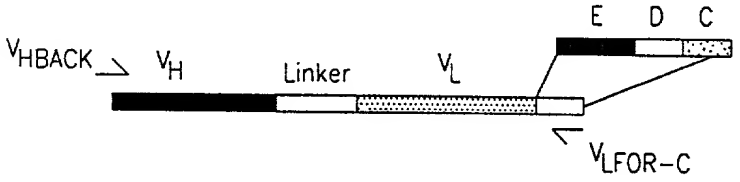
1. mRNA purification from spleen or PBLs

2. cDNA synthesis

3. amplification



4. assembly



V_H Primers

Oligo dT	HO–TTTTTTTT(T)$_n$ 3' 5'
V_{HBACK}	V_H back –OH 5' 3'
V_{HFOR}	J_H for HO– 3' 5'

V_L Primers

V_{LFOR}	J_{kappa} for HO– 3' 5'
V_{LBACK}	V_{kappa} back –OH 5' 3'
$V_{Linkers}$	J_{kappa} for E D C HO– 3' 5'
V_{LFOR-C}	C HO– 3' 5'

FIG. 7

Searching a recombinant antibody library

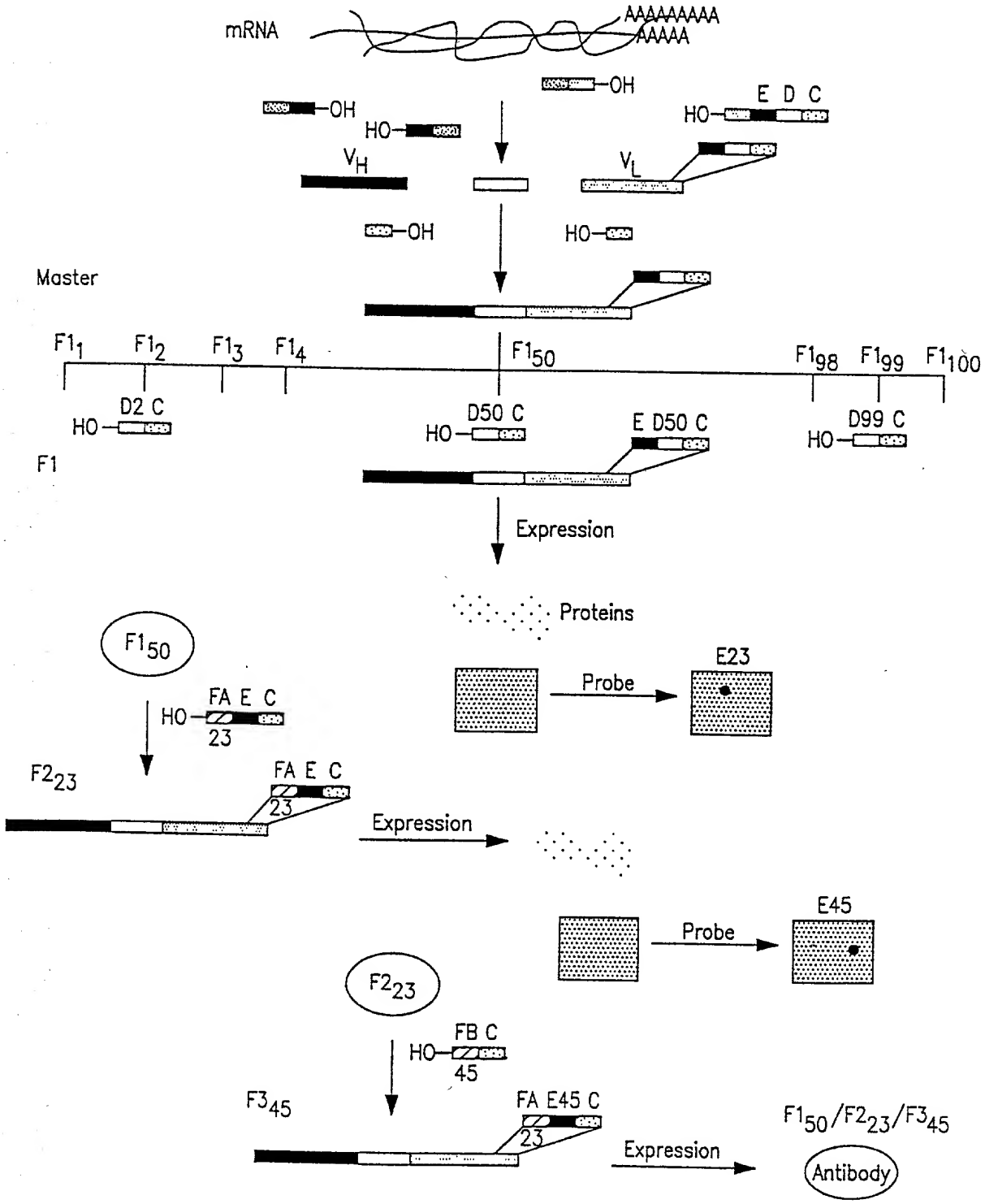
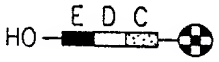


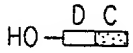
FIG. 8

Physical elements to include in the kits and combinations

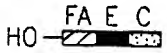
- *Anti-tag Arrays*™
- Primer sets



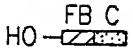
$D_1 - D_{1,000}$



$D_1 - D_{1,000}$



$FA_1 - FA_{1,000}$

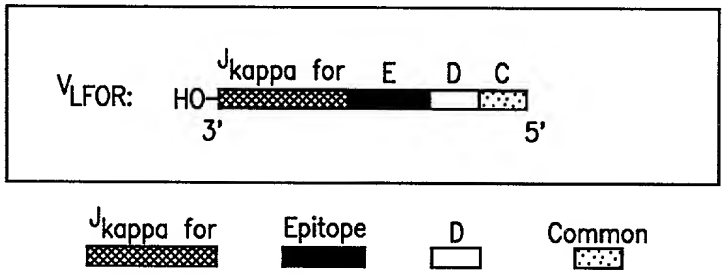


$FB_1 - FB_{1,000}$

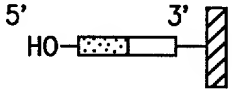
- Readers
- Software

FIG. 9

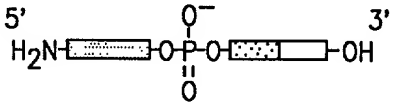
Making the V_{LFOR} primers: Solid phase synthesis



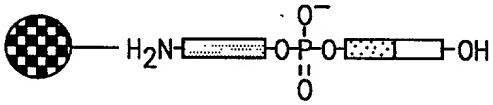
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extended by hybridizing with DNA patch and ligating

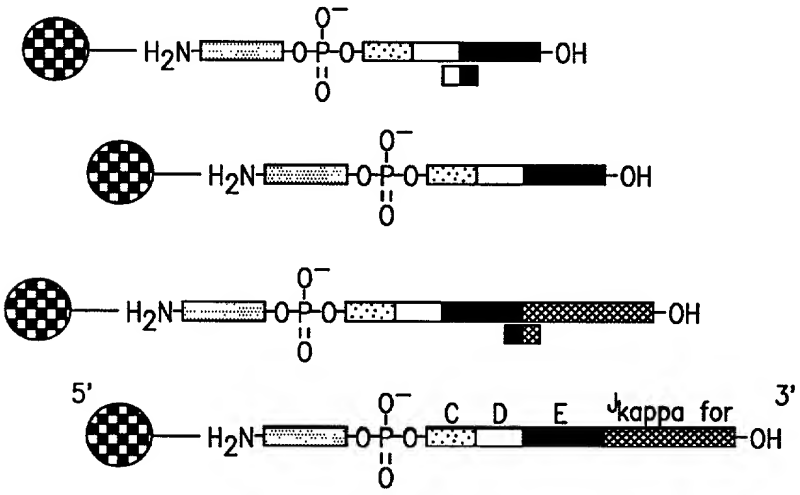
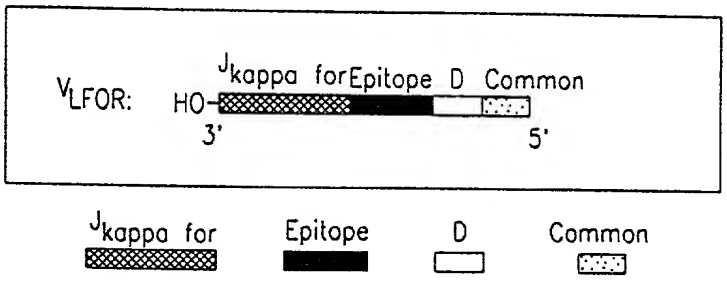


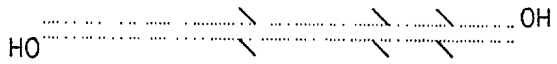
FIG. 10

Making the V_{LFOR} primers: Overlapping hybridiation

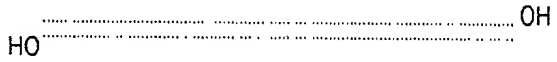


- Synthesize 4,028 different oligos:
  (26 for $J_{kappa\ for}$; 2,000 for Epitope, 2,000 for D; 2 for Common

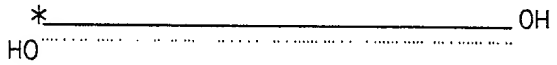
2. Assemble oligos for + and − strands of the different regions



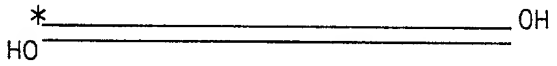
3. Ligase the assembled oligos



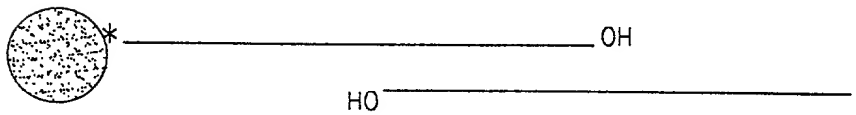
4. 1st strand synthesis with biotinylated primer



- 2nd strand synthesis with non-biotinylated primer



6. Bind to avidin coated magnetic beads and then denature



7. Purify non-biotinylated ssDNA

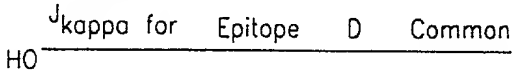


FIG. 11

Building the collection of antibody/tag pairs: Hybridoma screening

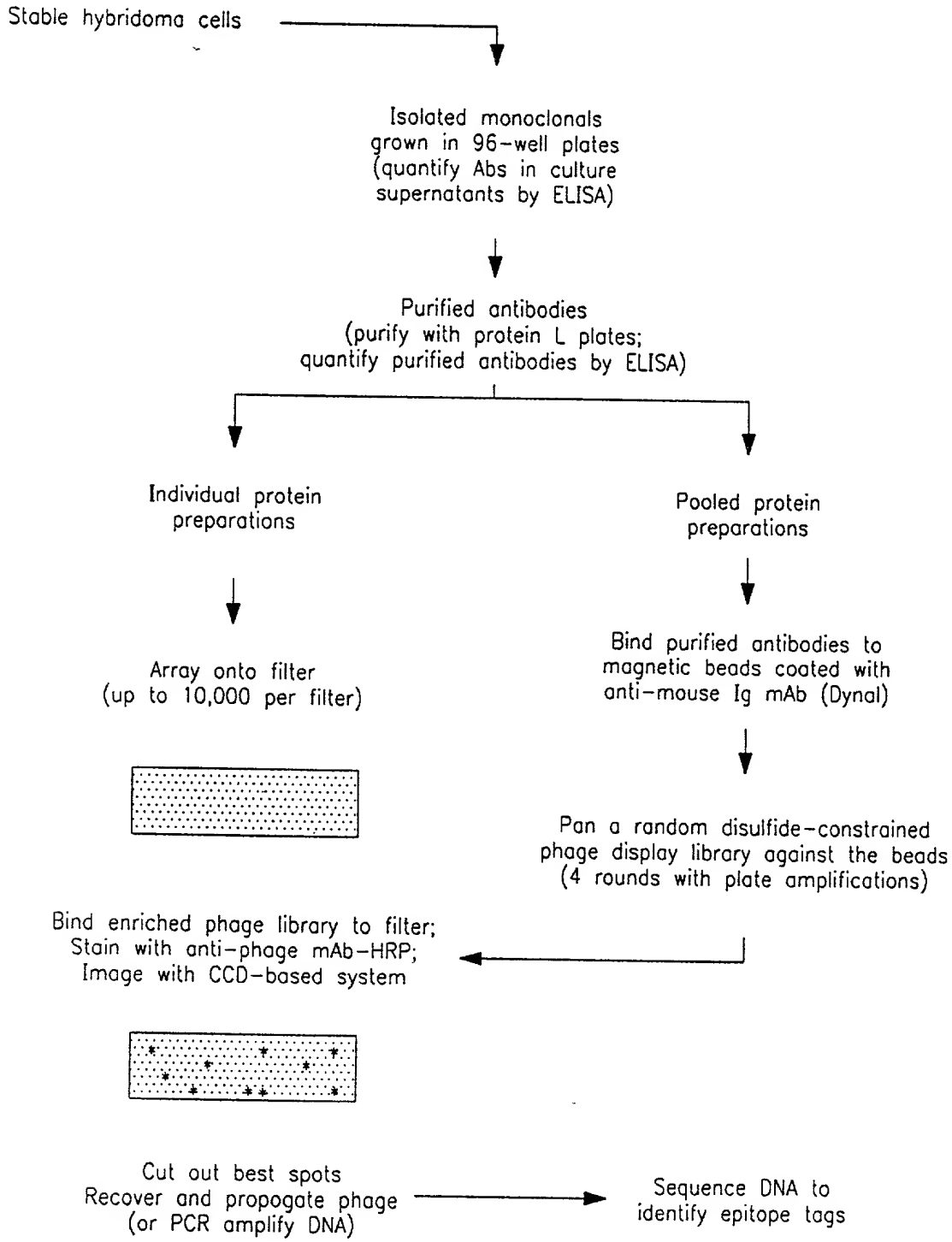


FIG. 12

Table 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR

A. Human VH back primers (sense)

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Human JH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuVk1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuVk2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuVk3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuVk4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuVk5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
HuVk6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuVλ1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
HuVλ2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuVλ3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuVλ4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuVλ5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuVλ6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human J kappa forward primers (anti-sense)

HuJk1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJk2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJk3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJk4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJk5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human J. lambda forward primers (anti-sense)

HuJλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

FIG. 13A

2. Linker fragment PCR

F. Reverse JH for scFv linker (sense)

	FR4 heavy / linker
RHuJH1-2	5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
RHuJH3	5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'
RHuJH4-5	5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
RHuJH6	5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'

F. Reverse Vk for scFv linker (anti-sense)

	FR1 light / linker
RHuVk1aBACKFv	5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'
RHuVk2aBACKFv	5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
RHuVk3aBACKFv	5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
RHuVk4aBACKFv	5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'
RHuVk5aBACKFv	5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'
RHuVk6aBACKFv	5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'

F. Reverse Vλ for scFv linker (anti-sense)

	FR1 light / linker
RHuVλBACK1Fv	5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
RHuVλBACK2Fv	5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
RHuVλBACK3aFv	5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
RHuVλBACK3bFv	5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'
RHuVλBACK4Fv	5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'
RHuVλBACK5Fv	5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
RhuVλBACK6Fv	5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'

3. Pull-through primers for introduction of restriction sites*

G. Human VH back (Sfi)primers (sense)

	FR1 heavy
HuVH1aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5uBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'

H. Human J kappa forward (Not) primers (anti-sense)

	FR4 light
HuJk1FORNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJk2FORNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAG CTT GGT CCC-3'

H. Human J kappa forward (Not) primers (anti-sense)(continued)

	FR4 light
HuJk3FORNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJk4FORNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJk5FORNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3'

H. Human J lambda forward (Not) primers (anti-sense)

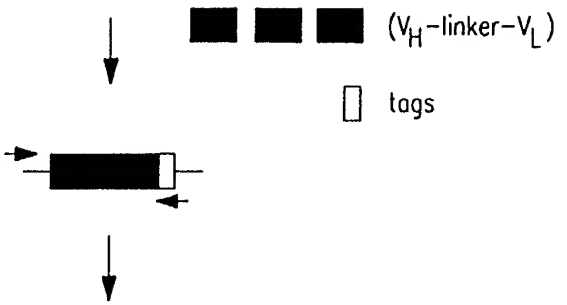
	FR4 light
HuJl1FORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJl2-3FORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJl4-5FORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAA AAC GGT GAG CTG GGT CCC-3'

*Recognition site for restriction enzyme is underlined.

FIG. 13B

step I

Tag and assemble immunoglobulin genes



Create 1,000 sub-libraries by separate PCR amplification reactions using tag-specific PCR primers

1,000 sub-libraries

FIG. 14A

step II

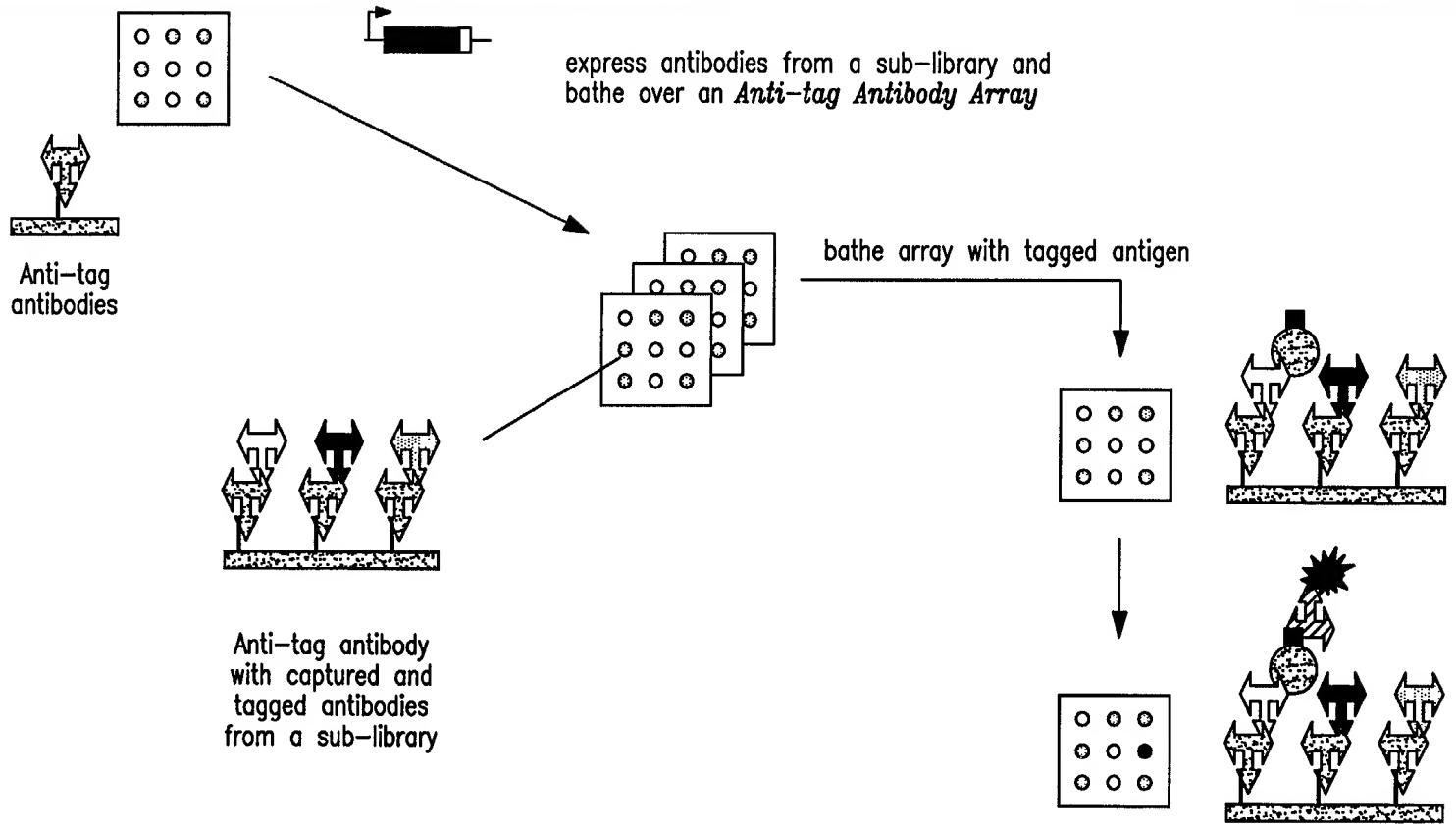
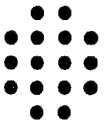
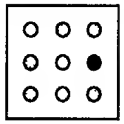


FIG. 14B



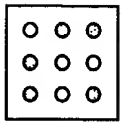
step III



Amplifiy the antibody genes from the identified sub-library using tag-specific PCR primers

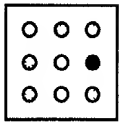
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs

Express and purify the antibodies



Re-distribute over an ***Anti-tag Antibody Array***

If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb

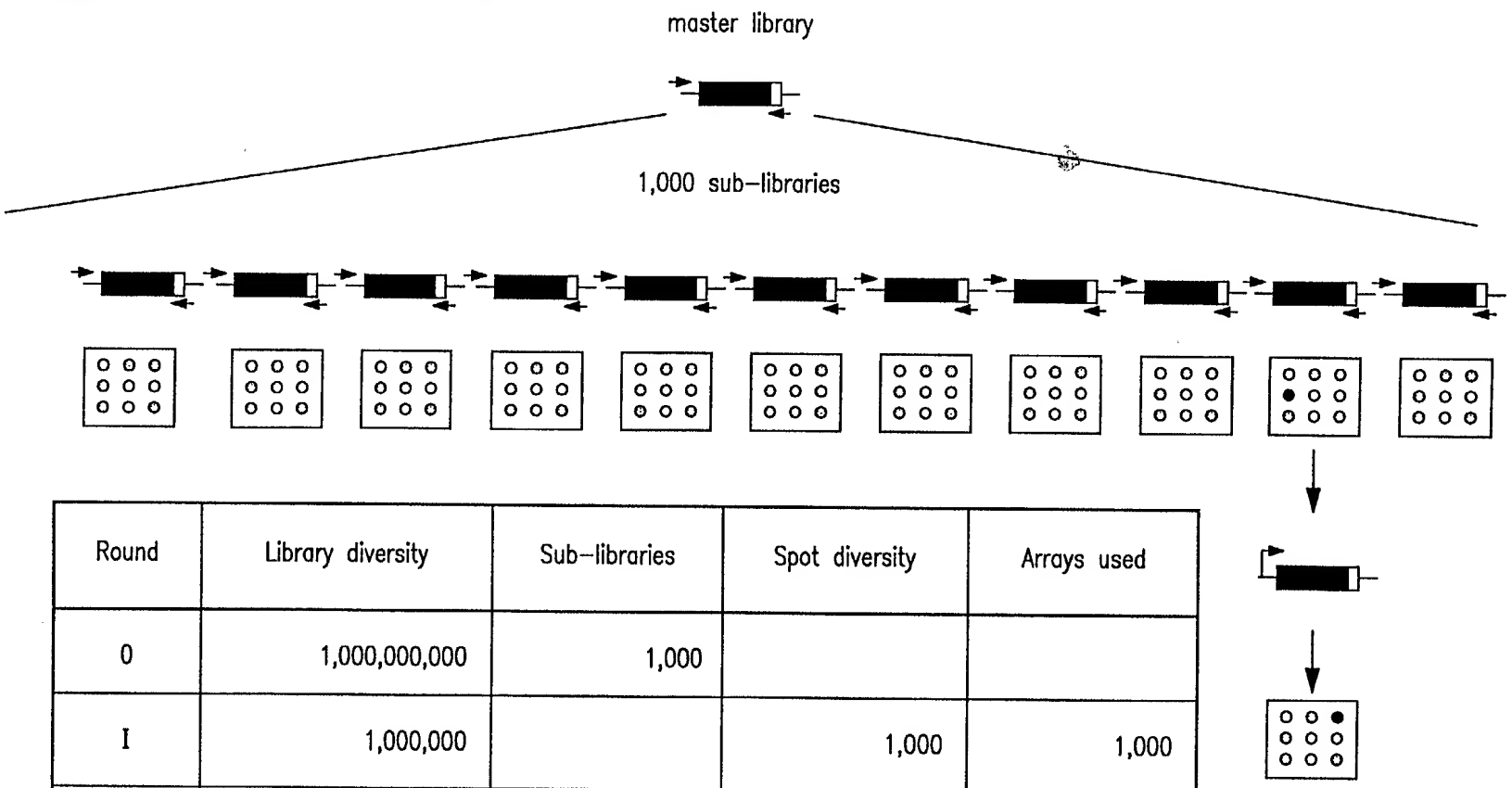


Re-survey to ID the antibody of interest

FIG. 14C

HELLER EHRMAN WHITE & MCAULIFFE LLP
Sheet 17 of 23
Title: COLLECTIONS OF BINDING PROTEINS AND TAGS AND USES THEREOF FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING.
Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751

summary



Round	Library diversity	Sub-libraries	Spot diversity	Arrays used
0	1,000,000,000	1,000		
I	1,000,000		1,000	1,000
II	1,000		1	1

FIG. 14D

Modification searches

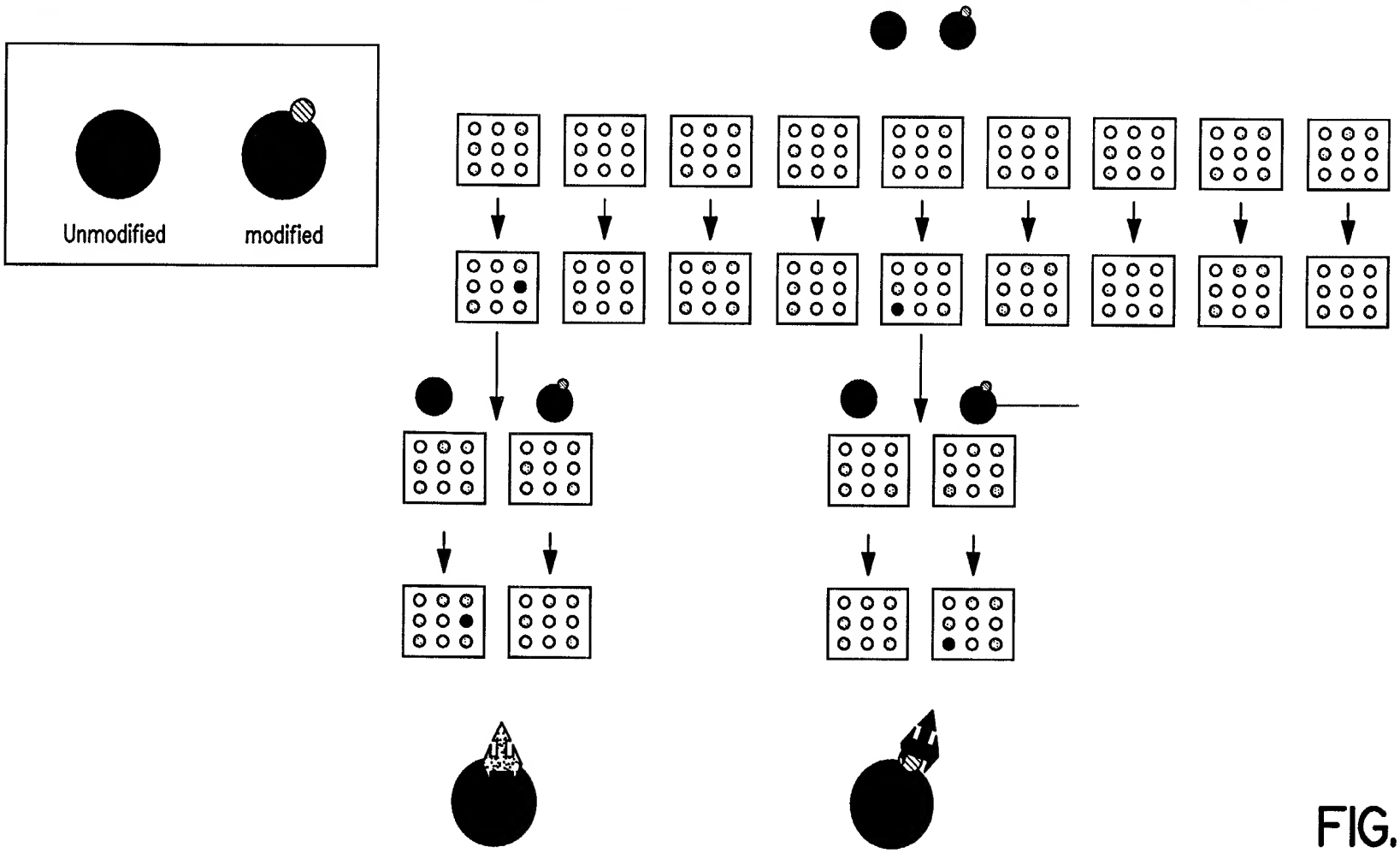
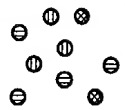


FIG. 15

Simultaneous searches



Round Arrays Bait Probe

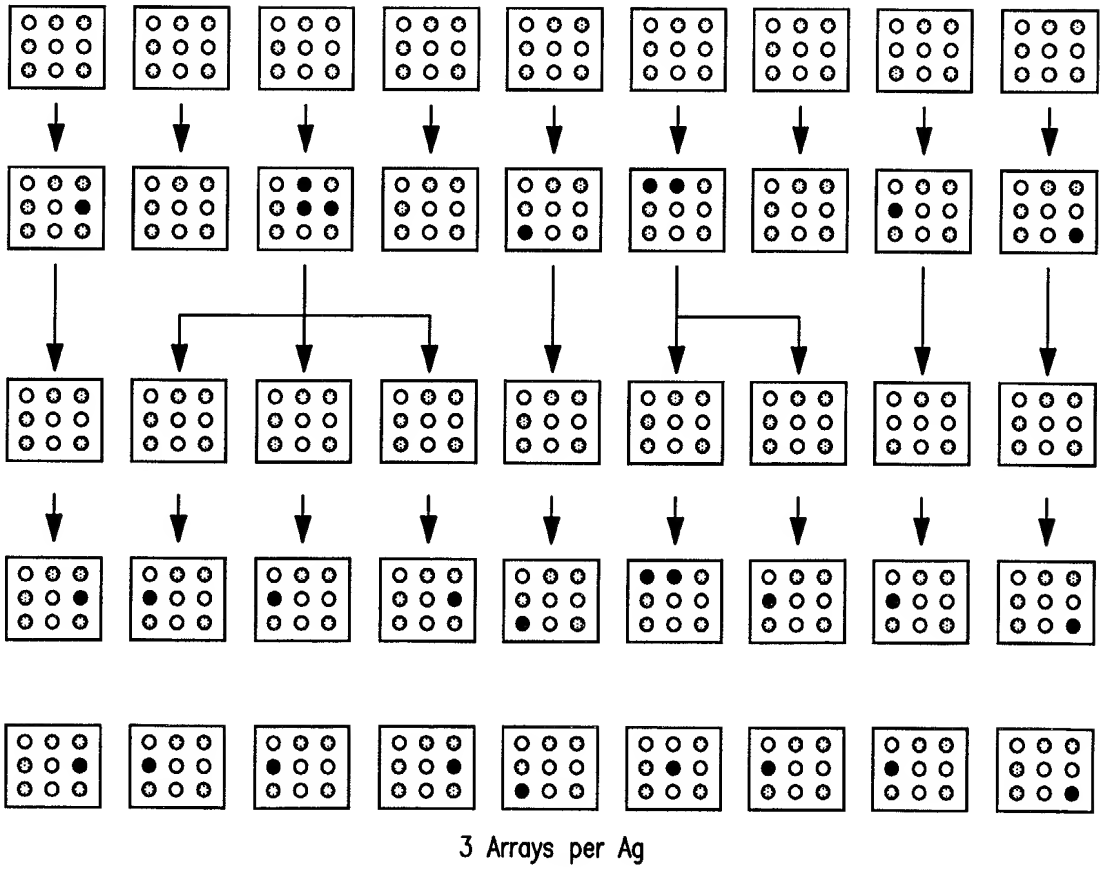
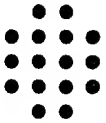
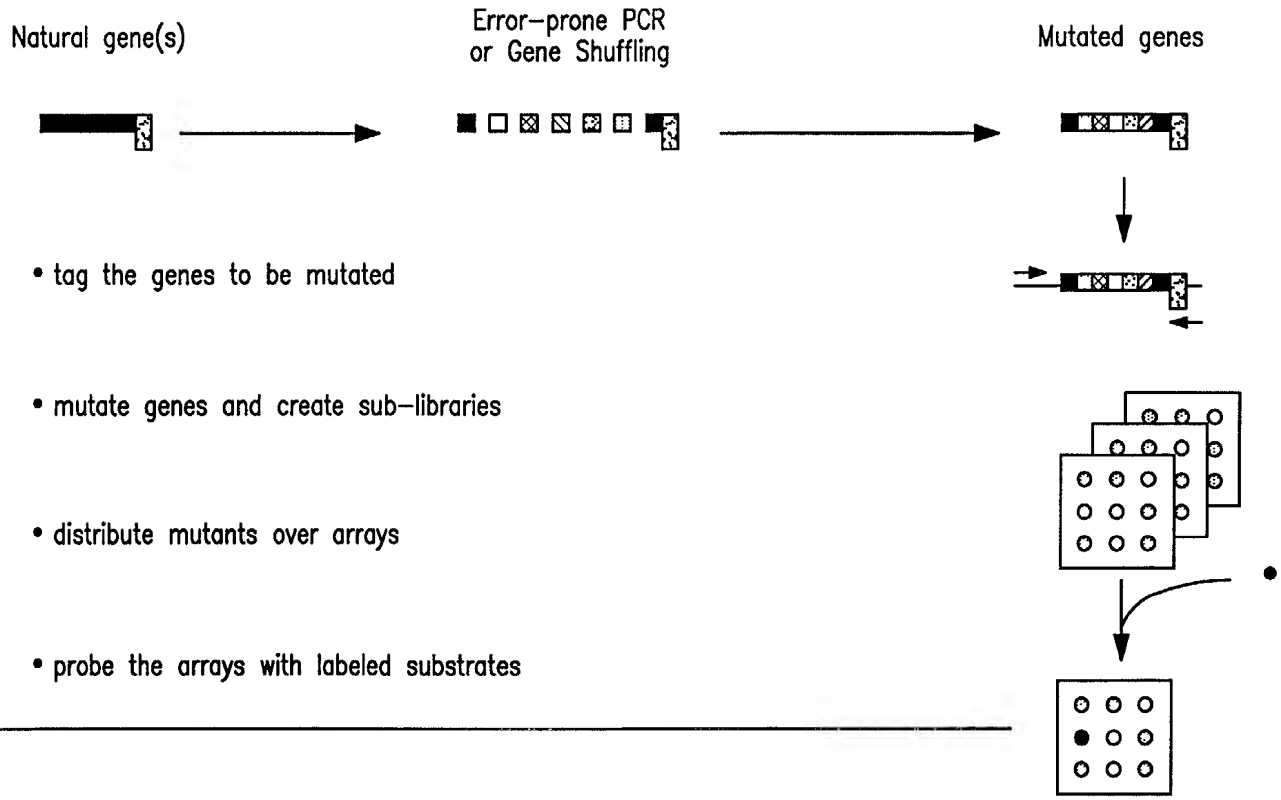
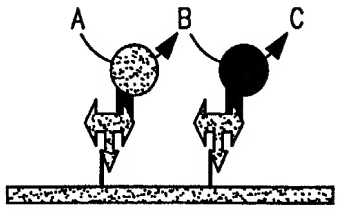


FIG. 16



Protein interaction mapping





Spots can contain mixtures of enzymes
for detection or pathway engineering

FIG. 17

Protein interaction mapping

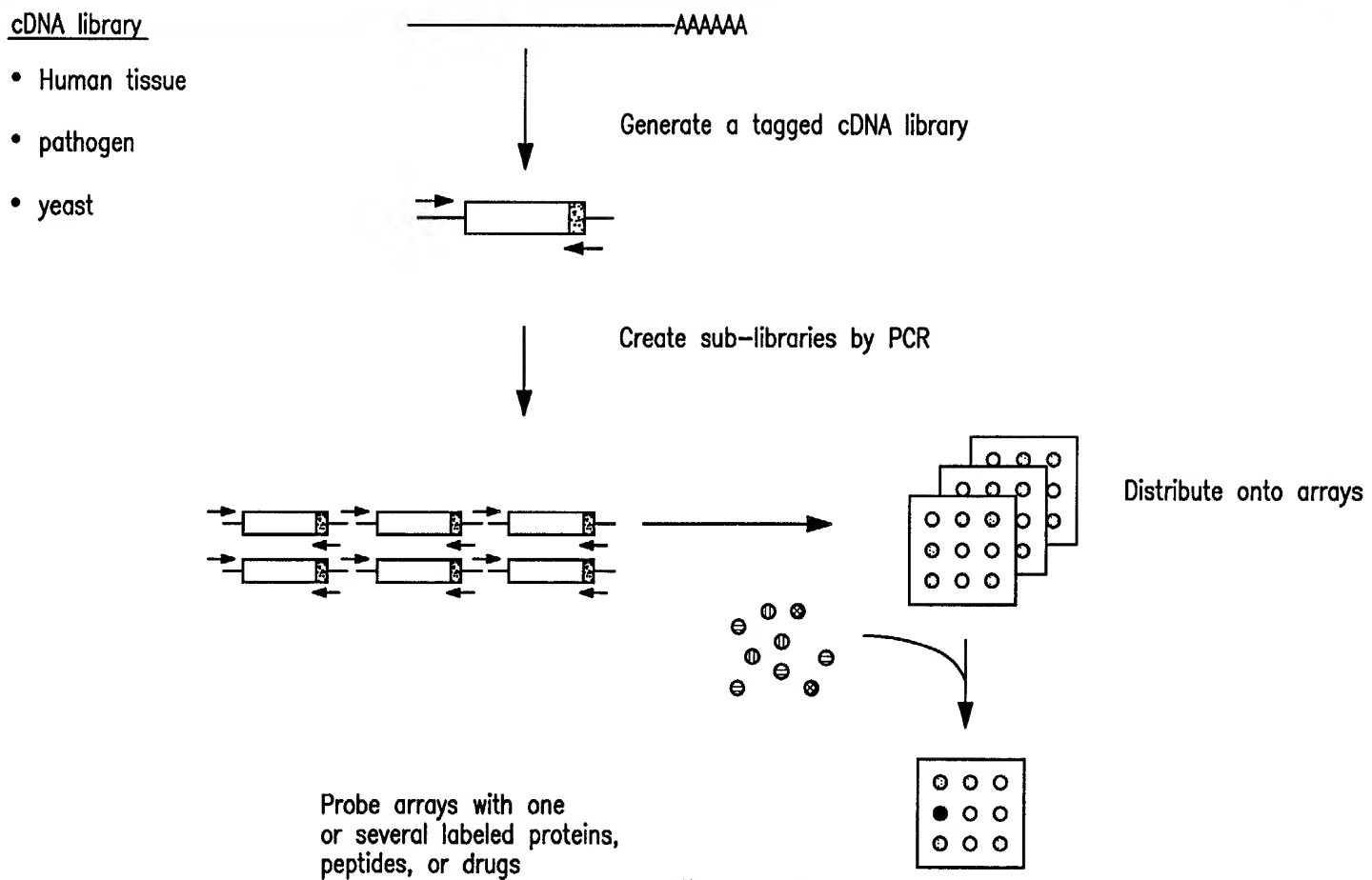


FIG. 18

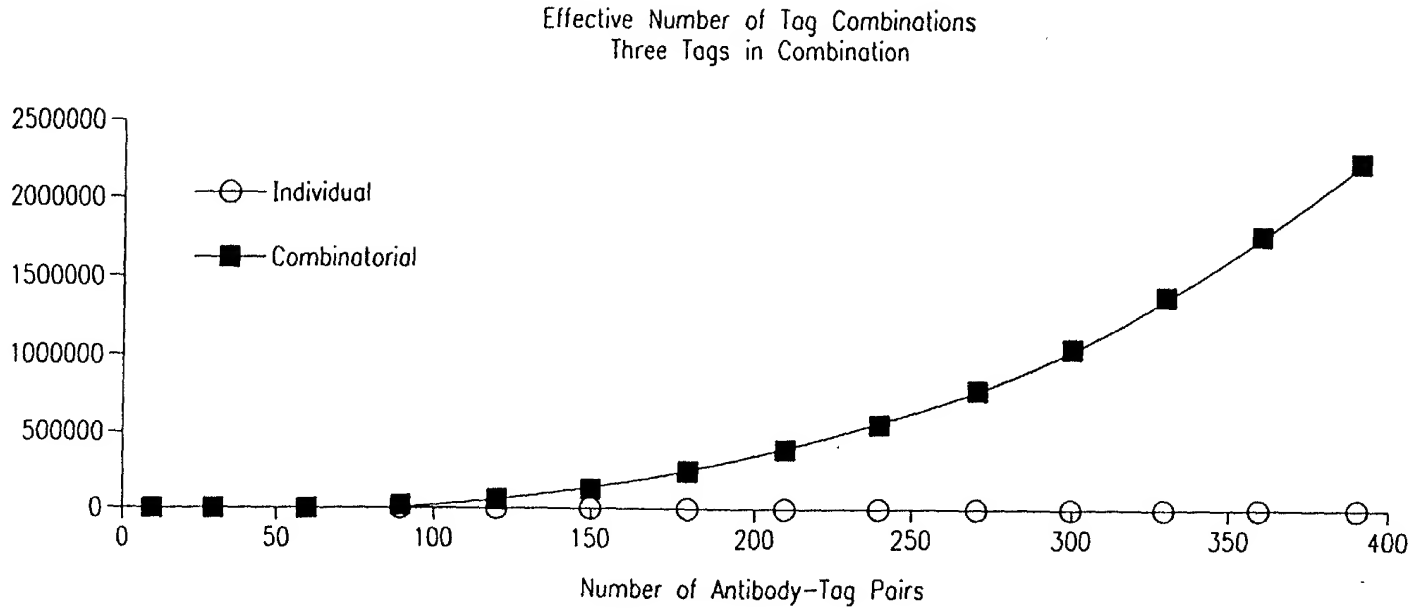


FIG. 19